Chapter 10 · Microbial Succession During the Decomposition of Organic Matter

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10.1 Introduction

One of the main ecological roles of the heterotrophic microflora is the decomposition of organic matter. By this means the essential nutrient elements, such as nitrogen, phosphorus and sulphur, are transformed from organic to inorganic forms and become available once again to primary producers. Another ecologically essential concomitant of microbially mediated decomposition is the formation of recalcitrant organic molecules, such as humic substances, which contribute to the chemical stability of the ecosystem.

The decomposition of any unit of organic matter, whether it is a seaweed frond, a tree stump, a faecal pellet or a paper bag, is almost invariably due to the activity of a diverse community of microorganisms and invertebrate animals. A particular feature of the interrelationship of the member species of these communities is that they commonly exhibit a succession. This phenomenon has attracted the attention of microbial ecologists, probably because it is thought to provide insight into a number of ecological phenomena of fundamental interest. These include: interspecific competition and other microbe–microbe interactions; the physico-chemical environment as a regulator of distribution and activity; the influence of chemical modifiers, such as antibiotics, on microbial activity; the substrate relationships and capacities of microbial species; dispersal and colonisation patterns; and other processes. In practice, the holistic nature of the successional phenomenon, embracing as it does such a wide range of community determinants, commonly eludes all attempts to interpret the changes observed.

Thus there have been a large number of studies of microbial succession on a wide range of organic materials (Hudson 1968; Dickinson & Pugh 1974; Frankland 1981) which have generated nearly as many theories of the fundamental basis for the patterns involved. These are fully discussed in the reviews quoted above and relevant theoretical material can also be found in Margaleff (1968), Odum (1969) and Horn (1976). This is not the place to review the theoretical basis of microbial successions but it is essential to clarify the concepts somewhat. It is axiomatic to this volume that the understanding gleaned from any experiment is, to a considerable extent, determined by the limitations of the methods used. It is also clear that the choice of method and interpretation of results will depend on the conceptual framework within which the experiment is viewed. This makes it imperative that we define some terms and limitations to our concept of succession to avoid possible confusion. Indeed it is possible that the most important preparation for a study of succession lies not so much in pondering over the techniques available, for they are few, but rather in gaining a clear preconception of the nature of succession.

10.1.1 Types of Succession

We may distinguish between what may be termed ecosystem succession and decomposer succession with some of the main characteristics given in Table 1. Ecosystem succession is a familiar concept which is described and discussed in all textbooks of ecology. The successional pattern of the ecosystem is determined by the dynamics of the primary producers. Colonising plants grow and alter environmental conditions in such a way as to promote their replacement by successional species. The trend of succession is towards a climax which optimises primary production, species diversity and other ecosystem characteristics. A succession of heterotrophs also occurs which is primarily determined by the composition, diversity and productivity of the plant community. At the climax, ecosystem production (P) and respiration (R) are balanced ($P/R = 1$). This occurs whether the succession starts in conditions where P is in excess (autotrophic succession) or where R is greater than P (heterotrophic succession). Ecosystem succession of this type is within the purview of the microbiologist—for instance in studies of microalgal succession in lakes, streams, oceans or in experimental microcosms (Margaleff 1968; Gordon *et al.* 1969; Fogg 1977). This chapter, however, is concerned with decomposer succession whose characteristics are quite different (Table 1). There is a single input of energy to the system which therefore shows a continuous decline in energy content and (secondary) productivity, theoretically to a zero level. In practice decomposition always leaves some organic residue within the usual period of study of a microbial succession. Whilst generalisations have been made for ecosystem succession concerning such features of community structure as diversity and interaction these remain largely speculative for decomposer succession (Swift 1976; Frankland 1981).

10.1.2 Structure of Successional Studies

Succession, as most simply stated, can be defined as a change in the composition of the biological community with time. It is necessary to qualify this simple view of a sequence in a number of ways that enable the most appropriate choice of method to be made.

A varying picture of any microbial community is obtained when it is observed at different scales of resolution. This applies both to the spatial and the temporal intervals between sampling points. For

Table 1. Characteristics of different types of succession. Some features of early and late stages of succession in ecosystem and decomposer successions. List modified after Odum (1969) with those for decomposer succession based on the hypotheses of Swift (1976).

	Ecosystem succession		Decomposer succession	
Ecosystem attributes	Early stage	Late stage	Early stage	Late stage
Total organic matter	low	high	high	low
Mineral elements	mainly inorganic and extrabiotic	mainly organic and intrabiotic	mainly organic and immobilised	mainly inorganic and mineralised
Mineral cycles	open	closed	closed	open
Rate of nutrient flux—organism to environment	rapid	slow	slow	rapid
Nutrient conservation	low	high	high	low
Biochemical diversity	low	high	high	low
Species diversity	low	high	high	low
Internal symbiosis and homeostasis	low	high	high	low
Stability	low	high	high	low
Spatial heterogeneity of habitat	low	high	low	high
Niche specialisation	broad	narrow	narrow	broad
Size of organisms	small	large	large	small
Growth form	r-selected	k-selected	k-selected	r-selected
Life cycles	short, simple	long, complex	long, complex	short, simple

instance if the microflora on the leaf litter of a temperate woodland were analysed every summer, it is probable that no major yearly differences would be noted and no concept of succession developed. If the top layer of the litter was sampled every two or three months, however, a sequential change in the microflora would be observed; alternatively, sampling every year, but in different (annual) horizons would reveal a succession which was related but not identical (Fig. 1). Use of an inappropriate scale in space can also produce misleading sequences. Within any temperate wood, for instance, a sequence of fungal fruiting can be observed during the year. This is not strictly a succession, however, for the fungi may not be utilising the same food base nor in any way influencing one another. Thus it may be concluded that our definition of succession should include some reference to the change in composition of the community with time 'in the same site' or preferably 'on the same resource unit'. Resource here means the organic matter being decomposed (Swift 1976); for practical reasons, as argued below, the natural unit of the resource in question (a leaf, a twig, etc.) is usually the best scale for investigation.

Most studies of succession have been confined to single biological groups—fungi, bacteria, protozoa and other microorganisms. The decomposition of most resources is, however, due to the interaction of all these organisms plus invertebrate animals of a variety of phyla. The capacity to interpret successional changes in any one group may be limited by the information available on other groups—a point which has been all too commonly ignored, particularly by mycologists.

The classical view of autotrophic succession pictures one set of species replacing another which eventually becomes extinct. In microbial ecology the concept of extinction is less commonly utilised; what is usually described is the changes in relative activity or abundance of species which are commonly coexistent over a considerable time span. The succession is, therefore, one of change in dominance rather than absolute presence and/or absence. In some microorganisms—fungi, protozoa, spore-forming bacteria, actinomycetes—periods of dominance or suppression may coincide with physiomorphs, e.g. mycelium as opposed to resting spore. This may in fact represent an overlapping sequence of life cycles with organisms going through phases of dormancy (as spore) and vegetative growth (plus reproduction in some cases) followed by dormancy again (Fig. 2). The different types of successional patterns that these produce may be paralleled in ecosystem succession by what Horn (1976) has termed obligatory successions and competitive hierarchies.

Whichever pattern is demonstrated, and in many cases presumably both can be present, the concept that successional species will influence one another is intrinsic to successional studies, i.e. that the colonising species will create conditions conducive to the growth of a succeeding species, the growth of which may inhibit the further activity of its

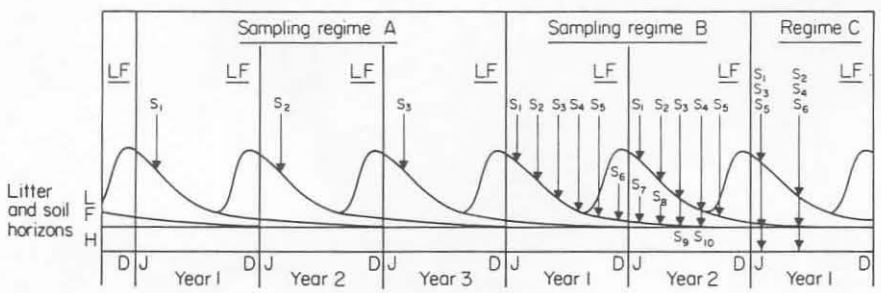

Figure 1. The importance of scale in the design of a sampling programme. The diagram shows three sampling programmes for leaf litter microorganisms which would result in differing conclusions concerning the pattern of community change with time. The programme is imagined as occurring in a forest ecosystem in which the leaf litter input occurs at a single time during the year (LF). The arrows indicate the dates and sites at which sampling for leaf litter microorganisms is carried out. (a) Annual sampling of surface litter three months after litter fall—community composition constant. (b) Sampling of litter layer at two-month intervals following litter fall—reveals a successional change in the leaf litter microflora community. Note that after litter fall in year 2 sampling may be repeated on new litter or may be continued on the same, but buried, layer. (c) Single sampling of different litter and soil horizons three months after litter fall reveals some aspects of the same succession. Further details in text.

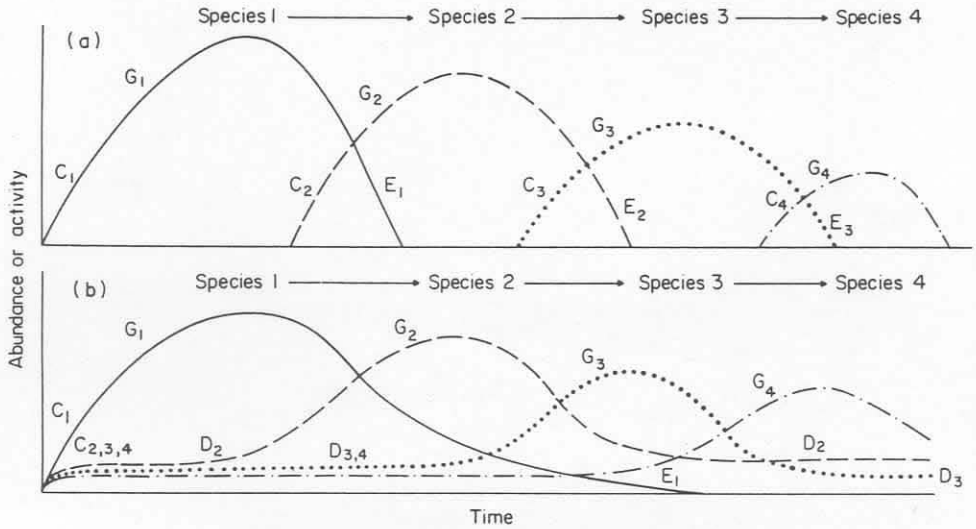

Figure 2. Possible patterns of succession. (a) Succession by species replacement and extinction (obligatory succession—Horn 1976). (b) Succession by change in species dominance (competitive hierarchy—Horn 1976). (C = colonisation phase; G = phase of active growth; E = extinction; D = dormancy; Species 1 = ———; Species 2 = — — —; Species 3 = · · · ·; Species 4 = —·—·—). For further details see text.

predecessor in some way. Thus studies of succession must utilise techniques for determining changes in the chemical and physical state of the resource as well as in the composition and activity of the decomposer community.

Experimental

10.2 Introduction

The study of microbial succession involves little in the way of specialised techniques—the main methods employed are those of observation, isolation and identification described in earlier chapters. What is important, however, is the experimental design of any study. The following account, therefore, concentrates on matters that determine the nature of a successful experimental plan.

We may recognise four distinct problems of method in the study of succession:

(1) the selection and placing of the resource in the environment of study;

(2) the determination of change in the resource after recovery;

(3) the determination of change in the microbial community inhabiting the resource;

(4) the elucidation of the factors regulating the observed changes.

Each of these aspects is dealt with in separate sections below but it should be realised that the choices of method made for the solution of one problem may influence and often constrain the choices relating to secondary problems.

10.3 Choice and placement of resource

The essence of succession studies is to recover and characterise at time T_n a resource unit which was labelled and characterised at time T_o. An immediate problem is to ensure that the initial labelling and characterisation is carried out with minimal disturbance of the resource or the environment in which it is placed. A very wide range of resources has been commonly employed in studies of succession and the choice is usually determined by the overall objectives of the investigation in hand. It is useful, however, to note some aspects of the choice of resource which may affect the results obtained. A distinction may be made, for instance, between the resources employed for study of natural successions and experimental successions. This distinction relates to the aim of the study: the objective in studying natural successions is to follow as accurately as possible a succession which occurs as a commonplace process within the natural environment whereas an experimental succession study is

one which either employs a manufactured or treated resource (e.g. purified cellulose or pesticide impregnated leaves) or places a natural resource in an environment that it would not normally occupy (e.g. cut timber placed in contact with the soil).

Among the natural resources that have been studied are a wide range of plant litters (leaves, twigs, branches, roots, reproductive structures—Hudson 1968; Dickinson & Pugh 1974; Swift 1977; Hayes 1979). Much more limited attention has been given to animals, but faeces (Webster 1970; Lodha 1974; Wicklow 1981), feathers (Pugh & Evans 1970) and hair (Griffin 1960) have been studied as well as carcasses (Putman 1978). Semi-natural resources include compost (Chang & Hudson 1967) and sewage (Sykes & Skinner 1971; La Riviere 1977) and various foodstuffs (Skinner & Carr 1976). Experimental resources include cut timber in contact with the ground (Butcher 1968; Käärik 1974) and in marine and freshwater environments (Jones 1974), cellulose (Tribe 1966), chitin (Okafor 1966) and lignin (Jones & Farmer 1967).

10.3.1 Unit Character of Resource

The character of the resource is essentially a sampling problem and any sample taken at a particular time must be replicated. The unit of replication may affect the results obtained. A common method of replication is to take standard weights or sizes of resource (e.g. in studies of leaf litter decomposition, litter bags have standard initial weights of either entire leaves or of standard diameter discs cut from leaves) but vital information may be lost by this approach. All resources have natural units (e.g. individual leaves, twigs, faecal pellets, carcasses). The microbial community of one unit may differ from that of another and this variation may be due to natural features of variation in the units or to opportunistic variation in the microbial colonisation of the units. The former variation embraces such factors as the difference between sun and shade leaves; differences in leaves shed at different times of the year; the effect of the diameter of a twig or branch on its rate of decay; and differences in composition of faecal pellets related to the feed or physiological state of the producing animal. Opportunistic variation defines the effects of differences in time and space of the population structure of colonising propagules which may result in identical resources receiving different colonising microfloras. Where detailed insight is required into the structure of the microbial community and the factors determining it the natural unit should provide the basis of replication. Furthermore, either natural or arbitrary limits may be set to the variation, for instance, by defining diameter classes in studying twig, branch or root decay (Swift *et al.* 1976).

Standardisation of unit size may be experimentally desirable. Where this is done it should be by the selection of approximately equal units (e.g. whole leaves) from a natural population rather than by cutting leaf discs or stem lengths of equal size as has been done in a number of studies. Cutting results in freshly exposed and damaged internal cells which lose nutrients and may have selective effects on the microflora.

For many experimental resources natural units do not exist. In these cases the size and nature of the unit should be chosen with care; for instance in studying the colonisation of wood blocks by freshwater fungi, Sanders and Anderson (1979) showed that the species diversity of the fungal communities was related to the size of the block. A number of purified resources can only be obtained in powdered or particulate form (e.g. chitin or lignin). Presentation to the microflora may in these cases involve suspension in some form of carrier material such as kaolin (Jones & Webley 1968). Care must obviously be taken to ensure that the carrier does not have any selective effect on the microflora. Some resources may be available in alternative forms in terms of their chemical composition or other characteristics (e.g. cellulose may be in the form of textile fibre, paper, cellophane and other forms, all differing in degree of crystallinity and polymerisation). Again the choice must be made with care and always bearing in mind the objectives of the study.

Further information on aspects of choice in relation to specific resources can be obtained from the general references quoted earlier. A general discussion of the way in which the characteristics of a resource (the resource quality) affects the rate of decomposition and the nature of its occupying microflora is given by Swift *et al.* (1979). The unit community concept is discussed by Swift (1976) and the problems associated with particulate organic matter are discussed by Fenchel and Jørgensen (1977).

10.3.2 Initiation and Termination

One of the most difficult aspects to elucidate when studying natural successions is when and by what microbes succession is initiated. In natural successions the actual line of initiation may be a matter of definition by the experimenter; thus all living plant parts have a natural surface microflora (e.g. Parkinson 1967; Dickinson & Preece 1976). Members of the surface microflora are often amongst the first organisms to be detected within the leaf or root during early stages of decomposition. Thus the problem arises of distinguishing between organisms which are incidental and superficial and those which are components of an inhabitant decomposer microflora. The same problem stated another way is that of determining when decomposition starts. Leaf death and decomposition is commonly initiated by pathogens (Hudson 1968) and these may, therefore, be defined as primary colonisers and initiators of succession. In other primary resources, however (twigs and fine roots, for instance), death may be more closely related to physiological variations within the plant itself and thus precede microbial colonisation.

In other resources the problem is somewhat different. Faecal pellets have a characteristic flora immediately after defecation which is quickly succeeded by other microbes. It is also preceded by a succession of microbial communities during passage of the food material through the gut of the animal. The decomposition of plant litter in fresh or marine water is brought about by a microflora which is quite different from that of the terrestrial organisms which may colonise the litter prior to its deposition in water (Jones 1974; Willoughby 1974). In all these cases the investigator must determine what the significant initiation point should be on the basis of the objectives of the investigation concerned. Techniques for the collection of plant litter (litter trapping) are reviewed by Newbould (1969), Chapman (1976) and Swift *et al.* (1979). Techniques for the collection of other resources, such as faecal pellets, can be obtained from the specific references given earlier. In experimental successions these problems are avoided—the experimental resource is generally freed of microbes and succession obviously starts at the time when the resource is placed in contact with a potentially colonising microflora.

Defining the end-point of a succession may be even more difficult. The decomposition of different resources tends to converge in the terminal stages. Thus leaves, twigs, roots, microbial tissues, carcasses and faeces all contribute terminal residues to soil which may be considered together as humus. A problem may arise in distinguishing a specific resource and its inhabitant microflora from its surrounds in the terminal stages of decay. There may be some value in doing so, however, as the microbial and chemical characteristics of a humus particle may be different according to the primary or secondary resource from which it is immediately derived. Very little consideration has been given to this aspect of succession and decomposition.

10.3.3 Environments

Any attempt to simulate or follow a natural succession must ensure that the environment in which the succession takes place is the natural habitat of the process. The environment is important not only because of its physical character—temperature, humidity, light and other factors—but also its biotic content—the population of propagules of the potential colonising microflora. Thus, for instance, the initial stages of succession and decomposition of plant matter take place while the resources are still attached to the plant. Both leaves and branches are colonised before litter-fall; in the case of the latter up to 50% loss in weight may have occurred by this time. Studies in which freshly plucked leaves or branches are placed on the soil surfaces must, therefore, be regarded as experimental rather than natural. The corollary of this is that a dramatic change in environment, such as occurs at litter fall, dispersal of fruits, deposit of faecal pellets, submergence of aquatic detritus, or burial of carcasses, is an important event within any succession and merits particular study. These features, however, have been commonly ignored.

Information on the relationship of environmental factors to the patterns of succession may be obtained by the use of microcosms for successional studies. The best examples of this are those of autotrophic and heterotrophic ecosystem successions in freshwaters (Gordon *et al.* 1969; Cook 1977). Soil microcosms have also been used by a number of workers although this has been less common in recent years (Waksman & Cordon 1939; Dix 1964). Information on methods of manipulating soil physical conditions can be obtained from the review by Griffin (1972) and, for aquatic systems, from that by Golterman *et al.* (1978).

Although experimental successions by their very nature often employ unnatural combinations of resource and environment, care should be taken in the placement of the resource. In experiments on cellulose decomposition in soil, for instance, cellulose strips may be placed either vertically or horizontally in the soil profile depending on the question being investigated.

10.3.4 Labelling

The commonest way of labelling resource units or samples at time T_0 so that they may be recovered at time T_n is by confining them in a mesh bag or cage—a procedure usually termed the litter bag technique. Meshes of varying size may be employed: finer meshes have the advantage of confining litter so that little is lost even when it breaks up into relatively small particles in the terminal stages of decay; on the other hand, fine meshes prevent the entry of larger soil organisms. Swift *et al.* (1979) categorised the decomposer biota in terms of body diameter thus: macrofauna (earthworms, amphipods, millipedes, etc.) >2 mm; mesofauna (dipteran larvae, termites, collembola, acari, etc.) $>$ 100 μm; microfauna and microflora (protozoa, fungi, bacteria) <100 μm. The choice of appropriate mesh size can thus act as a selective agent for the biota.

Despite the very common use of the differential mesh exclusion technique in decomposition studies, it has not been exploited at all in studies of microbial succession. However, this would be a worthwhile approach for it would enable exploration of the effects of different animal groups on the patterns of microbial succession, a factor likely to be important. It is clear from recent studies that grazing by soil animals can alter the composition and activity of the decomposer microflora (Parkinson *et al.* 1978; Hanlon & Anderson 1980; Lussenhop *et al.* 1980). Similar effects may also occur in aquatic systems (Barlocher 1981).

The mesh size of an enclosure also affects the internal environment (e.g. fine mesh bags tend to retain moisture levels beyond that of unconfined litter). A comparison of the decay rate of confined and unconfined leaf litter by Witkamp and Olson (1963) showed that unconfined oak leaves decayed nearly twice as fast as leaves in a mesh bag that purportedly enabled the access of decomposers of all sizes. However, in a similar experiment Woodwell and Marples (1968) found only a small difference of rate in favour of the unconfined litter. The litter bag technique has been discussed by Gilbert and Bocock (1962), Chapman (1976) and Swift *et al.* (1979).

Larger resources, such as branches, twigs or roots, may be labelled by tethering them by a length of nylon thread to a marker post set in the soil. A similar approach has been used for leaf litter by some workers because of the environmental drawbacks of litter bags mentioned above. Variations in the enclosure technique have been used for other resources such as faeces or carcasses and in environments other than soil (see general references cited above).

10.4 Change in the state of the resource

Information relevant to the interpretation of a microbial succession may be obtained from the assessment of changes in the physical condition and chemical composition of the resource with time. In particular the rate of decomposition of the resource is a valuable parameter. Information related to chemical change may be particularly significant if a distinction can be made between the constituents of the resource and of the microbial biomass.

10.4.1 Establishing the Time Scale

The choice of the correct time interval for sampling may be of crucial importance for the type of microbial pattern revealed, as the comments in section 10.1 and Fig. 1 have explained. The discussion of initiation and termination given in the previous section is also relevant.

In some types of successional study it is possible to observe the same set of resource units at successive time intervals (Fig. 3a). An example of this is the observation of the sequence of fungal fruiting on dung (Harper & Webster 1964) or other resources. In most cases, however, the sampling techniques require that the resource unit be destroyed at the time of sampling (see below 10.5, section 4). Thus a replicated programme allowing for destructive sampling must be instituted. In most cases samples can be placed at T_0 and sampled at regular intervals (T_1, T_2, T_3, ... T_n) thereafter (Fig. 3b). In some cases, however, this is not practicable: for instance, the decomposition of wood may take five, ten or even more years from litter fall. An alternative to an experiment lasting

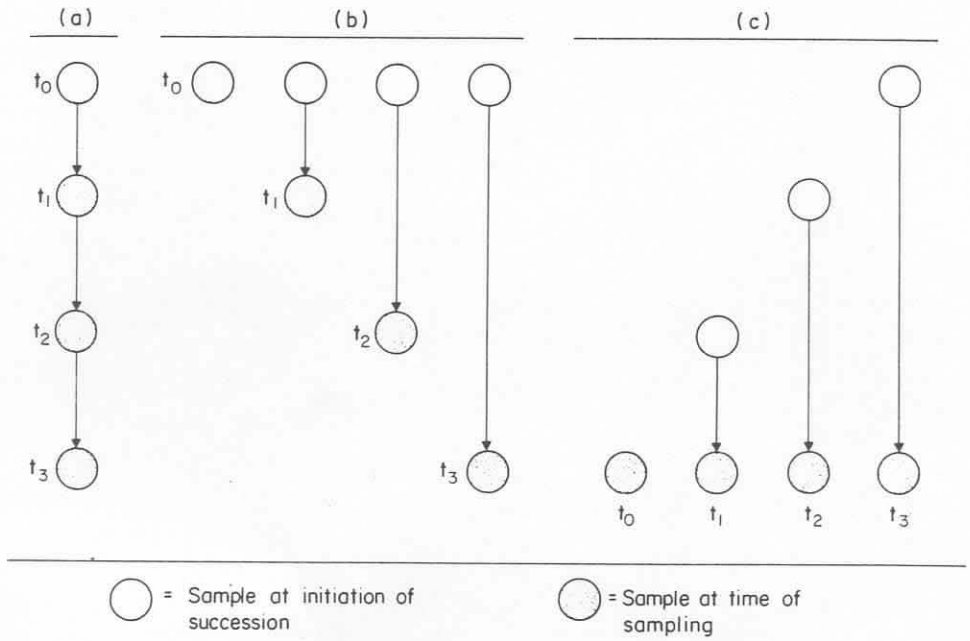

Figure 3. Different designs of sampling programme. (a) Sequential non-destructive sampling of the same sample of resources. (b) Sequential destructive sampling of replicated resource samples. (c) Contemporaneous destructive sampling of replicated resource samples in which succession was initiated at different times. In all cases replication of the sampling units within samples is not shown in this diagram.

this number of years is to obtain resources in different stages of decomposition (i.e. at different times from the start of decay) and analyse them contemporaneously (Fig. 3c). A similar approach may be used with leaf litter by sampling different horizons of litter and soil which represent litter inputs of successive years or seasons (see Fig. 1c—Visser & Parkinson 1975). In these instances it should be noted that an exact time base can rarely be established although an indirect estimate of the extent of decomposition may be possible (see next section).

10.4.2 Change in Physical State

Generally the most useful measurement of the change in physical state is to estimate the change in mass of the resource and thence the rate of decomposition. Two important problems associated with this are that of assessing the way(s) in which weight loss has occurred and that of separating the weight of resource from the biomass of the inhabitant microflora.

Weight may be lost from a decomposing resource by chemical transformation (catabolism) or by loss of chemically intact fragments (export). The latter is generally a physical process which is brought about by animal feeding activity. Animals either directly export the material within their intestines or their feeding produces material of small particle size (comminution) which then 'falls out' of the resource and its enclosure. As the microbiologist is usually more concerned with the direct catabolic effect of organisms on the resource it is useful to distinguish export losses from catabolism.

One approach is to ensure that the latter do not occur by confining the resource in a fine mesh enclosure, but this inevitably introduces both environmental and biological bias (section 10.3.4). If animals are allowed access to the resource, an approximate estimate of their activity can be made by assessing the change in volume of the resource. Thus tunnelling in wood can be measured by estimating the proportional area affected in successive cross-sections of a piece of wood. Then the volume of wood removed (T) can be calculated from:

$$T = \bar{A}.l$$

where $\bar{A}$ is the mean of the total area of tunnels

exposed in a series of cross-sections and l is the length of branch affected by the animals. Estimation of the total volume (V) and weight of the branch (m) gives the weight per unit volume of wood remaining, the relative density, d where:

$$d = m/(V\text{–T}).$$

By estimating d at successive time intervals the change in weight due to microbial decay (catabolism) can be estimated. A similar estimate can be made for planar resources, such as leaves or strips of cellulose, by using weight per unit area to allow for pieces removed by animal feeding. It must be recognised, however, that these estimates are approximate: firstly, not all animal feeding is measurable by these means, e.g. in leaves in addition to the pieces bitten out of the whole leaf and thus measurable as loss in area, there will be surface erosion which is not measurable; secondly, losses in weight per unit volume may occur by leaching as well as by catabolism. This, of course, is particularly important in aquatic environments. More detailed discussion of the relative importance of catabolism, comminution and leaching is given by Swift *et al.* (1979).

The distribution between microbial biomass and resource mass is also a technically difficult problem. The methods for estimating microbial biomass are reviewed in Chapter 6. The choice of method is to some extent determined by the resource type and organisms being studied but it should be noted that for this particular purpose general, non-discriminatory methods, such as ATP or hexosamine determinations, may well be suitable as the main purpose is to distinguish between microbe and substrate biomass. It will only generally be thought necessary to do this if detailed information on decomposition activity, particularly chemical change is required.

Care should be taken for possible weight inaccuracies due to the accumulation in the resource of extraneous material, such as mineral particles from soil. These should be brushed off before weighing. Washing off the material should be avoided as weight losses due to leaching can occur.

The choice of temperature for drying will be governed by the type of resource under study. Whilst high temperatures (100°C) are probably satisfactory for plant material, lower temperatures are necessary for soil and animal samples because of the presence of volatile materials. In some cases freeze-drying may be preferable. Allen *et al.* (1974, 1976) give advice in relation to specific materials.

In cases where non-destructive sampling is to be employed, indirect estimates of weight may be made by sacrificing replicates or sub-samples to estimate moisture contents. In cases where the time scale is not fixed estimates of the state of decay may be made by measuring the relative density of whole resources (Healey & Swift 1971; Swift *et al.* 1976).

10.4.3 Change in Chemical Composition

Chemical changes in the resource have commonly been invoked as important factors in determining the successional change in the microbial community. In particular the major components of the plant cell wall (cellulose, hemicellulose, lignin, etc.) have been suggested as regulators of activity in terms of the correlation of the patterns of chemical change with the apparent enzymic capacities of the successive groups of inhabitant fungi (Garrett 1963). Other authors (Hudson 1968; Swift 1976; Frankland 1981) have pointed out that these nutritional correlations are inadequate to explain the observed patterns of succession. Nonetheless, information on the patterns of chemical change in the resource are essential to the interpretation of the sequence of activity in the microbial community.

Methods for the analysis of both organic and inorganic constituents of plant tissues are given by Allen *et al.* (1974, 1976). Again the value of distinguishing microbial from resource components is emphasised (see Chapter 6, for a starting point for relevant references in this respect). Highly detailed analyses of cell wall constituents may now be made by use of sophisticated separative techniques (Keegstra *et al.* 1973; Montgomery 1982) and use may be made of these under some circumstances.

10.4.4 Calculation of Decomposition Rates

Chapman (1976) and Swift *et al.* (1979) both discuss the question of the appropriate form in which to summarise a decomposition rate. The most convenient and commonly utilised form is as a negative

exponential usually calculated from the fraction of weight remaining and is given by:

$$k = \frac{\log_e (W_T/W_o)}{T - T_o}$$

where k = decomposition rate; W_T and W_o are the mass of litter at the given time (T) and the initial time (T_o) respectively. Despite the general usefulness of this form of curve it should always be remembered that more insight into the pattern of resource change and to the biological activity that brings it about, may come from plotting the basic data as a decay curve than from summarising it as a single mathematical expression. The deviation from a standard format may be more instructive than agreement.

10.5 Community change

According to the strictest definition, succession takes place when species B is found to occupy a spatial location previously inhabited by species A but in which the presence of A can no longer be detected. In practice a change in relative abundance and/or activity of the two species in the same general habitat (i.e. on the same resource) is taken as evidence of succession (see Section 10.1). Thus an essential aspect of successional investigation is the identification of microbial species in microhabitats but methods for measuring abundance and activity are also pertinent. The secret of successful study and interpretation lies in the selection of the appropriate methods from the wide choice available. Detailed information on the methods and the criteria on which to base a choice are given elsewhere in this book (see Chapters 1 to 4) and will not be discussed here; a few fundamental points are, however, worth reiterating.

In most cases the only way of identifying the presence of a microbial species in a given habitat is by its isolation and culture under defined conditions. This is usually done by plating fragments of the resource onto an agar medium either with or without the use of pre-treatments, such as serial washing (see Chapter 2). As emphasised elsewhere in this book all such methods are necessarily selective. Choice of any particular isolation technique or medium may well run the risk of a failure to detect significant components of the microbial community. Thus a combination of media is generally desirable. Furthermore, isolation methods will fail to distinguish between active cells or hyphae and those which are dormant. Indeed, in the fungi there is a distinct risk that a species present in the form of dormant propagules, such as chlamydospores, may be preferentially isolated or enumerated on agar plates over a species present as active growing mycelium. Such methods commonly detect the past history of the resource rather than present activity.

Isolation techniques should be supplemented by direct observation. Microbial communities may be viewed under either the light or the electron microscope either directly or after some preparative manipulation, such as the sectioning of the resource (Jones & Griffiths 1964; Anderson 1978) or mounting it in some appropriate form. A range of techniques for the direct observation of soil microorganisms are reviewed by Parkinson *et al.* (1971). Aquatic organisms may be viewed simply in suspension or particle surfaces may be scanned (Meadows 1971) after appropriate sampling. Macroscopic fruiting in terrestrial fungi can be promoted by placing the resource in damp chambers and by subjecting them to a variety of environmental stimuli (Manachère 1980). Jones (1971) has described methods of promoting the sporulation and fructification of aquatic fungi.

Direct observation techniques suffer in general from the difficulties of positive identification: the cells or hyphae of different species may be indistinguishable from one another. On the other hand physiologically different forms of the same organism (e.g. vegetative cells or hyphae compared with dormant spores) may be detected. The use of vital stains in such preparations may give additional information on the viability and activity of microbial cells or hyphae (Babiuk & Paul 1970). Reproductive structures, particularly in the myxomycetes or true fungi, are identifiable and many detailed successions have been described on the basis of sequences in fruiting. As Harper and Webster (1964) pointed out, however, fruiting is only one of many activities in the life of a fungus and may be quite misleading in terms of presence or absence or indeed dominance.

10.6 Mechanisms of community change

Jacques Monod said that 'the study of the growth of bacterial cultures does not constitute a specialised subject or branch of research (but) covers

actually our whole discipline' (Monod 1949). The same could be said of the relation between the 'subject' of succession and microbial ecology as a whole. As emphasised earlier, the progress of succession subsumes a very wide range of ecological phenomena. The main value of the study of succession then rests in providing a structure for the investigation of these phenomena—competition, colonisation strategies, niche determination, environmental influences on these processes and many other facets. Conversely, the interpretation of a pattern of succession is dependent on isolating and identifying the individual factors which determine the observed changes in community composition and structure.

The usual approach to resolving these questions is to perform controlled experiments on individual species, or pairs or groups of species, selected from within the successional communities. The experiments are best performed in controlled environments simulating the conditions within which the successional changes take place and utilising resources or substrates which are chemically related to the various stages of resource change that occur within the natural succession. It would be impracticable and futile to list all the different types of experiment that have been carried out to resolve successional phenomena. Thus the references below merely seek to provide a broad cover of some of the more significant factors that have been investigated in the last twenty years or so.

The success of any organism in taking its place in a succession will depend both on its capacity and opportunity. The capacity of an organism is based in its genotype; the opportunity arises when that genetic potential is given phenotypic expression. The opportunity depends on the occurrence of an environment conducive to success and on the past history of the organism in the sense that it must be able to deploy sufficient intrinsic resources (e.g. colonising propagules, food supply and other factors). The spectrum of intrinsic factors which contribute to this success have been analysed by Garrett (1956, 1963) and summed up in two concepts 'competitive saprophytic ability' and 'inoculum potential' (see also Frankland 1981 for a good discussion of these). Garrett's scheme provided a great stimulus for research and many of the experiments mentioned below deal with one or more of the characteristics which he thought important. Garrett summarised a number of the studies carried out by himself and co-workers on the colonisation of wheat straw buried in soil (Garrett 1956, 1970).

Perhaps the most exemplary experimental study is that of Harper and Webster (1964) on the succession of fungi fruiting on rabbit dung. They studied mycelial growth rates, spore germination and time taken to fruit, in relation to the competitive influence both of the fungi and bacteria. They were able to demonstrate the complexity of the interactions involved in successional phenomena as well as discovering a new form of antagonism between hyphae. Growth rates and antibiotic effects were postulated by Garrett as two of the main features of competitive saprophytic ability and several studies have followed this up. Wastie (1961) and Gibbs (1967), for example, showed the possible importance of a toxic environment on succession in pine logs. The enzymic capacity of organisms is also an important feature of their ability to occupy a particular resource at a particular time; many authors have explored this with laboratory tests (Flanagan & Scarborough 1974) or inoculations into litter and subsequent analysis (Hering 1967), or a combination of the two (Frankland 1969). Environmental aspects have less commonly been considered: Hogg (1966) transferred leaves between different soil enviroments to investigate the effect on the pattern of succession and also measured spore viability in the soil.

The 'opportunistic' feature of succession is more difficult to experiment with but the importance of seasonality in relation to the air spora has been demonstrated by both Meredith (1959) and Dowding (1969) for somewhat different woody resources. The ideas of Garrett (1970) on the inoculum potential of propagule populations could be usefully incorporated into successional experiments of a similar type to those involving plant pathogens (Wastie 1962; Chou & Preece 1968). Experiments into inter-specific competition have been carried out by inoculating pairs or groups of species into natural habitats or resources (Harper & Webster 1964; Rayner 1978).

The examples above are concerned largely with fungi. The manipulation of bacteria and protozoa in pure culture is aided by the possibility of utilising continuous as well as batch culture systems (Veldkamp 1977; see also Chapter 20). It should be noted, however, that the choice of continuous culture versus batch culture should be made with care, bearing in mind the type of system being simulated (see Chapter 1).

This is an incomplete and selective list of the types of experiments that have been carried out to assist the interpretation of successional phenomena. Reference to the papers mentioned should, however, help to clarify possible approaches to the problems of method that are encountered. Nonetheless it should be said that there has been a sad lack of ingenuity in such experiments in the last fifteen years and the time is ripe for fresh approaches.

Recommended reading

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